2,5′-Disubstituted Adenosine Derivatives: Evaluation of Selectivity and Efficacy for the Adenosine A_1, A_{2A}, and A_3 Receptor

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Novel 2,5′-disubstituted adenosine derivatives were synthesized in good overall yields starting from commercially available guanosine. Binding affinities were determined for rat adenosine A_1 and A_{2A} receptors and human A_3 receptors. E_{max} values were determined for the stimulation or inhibition of cAMP production in CHO cells expressing human adenosine A_{2A} (EC_{50} values as well) or A_3 receptors, respectively. The compounds displayed affinities in the nanomolar range for both the adenosine A_{2A} and A_3 receptor, without substantial preference for either receptor. The derivatives with a 2-(1-hexynyl) group had the highest affinities for both receptors; compound **4** (2-(1-hexynyl)adenosine) had the highest affinity for the adenosine A_{2A} receptor with a K_i value of 6 nM (A_3/A_{2A} selectivity ratio of approximately 3), whereas compound **37** (2-(1-hexynyl)-5′-*S*-methyl-5′-thioadenosine) had the highest affinity for the adenosine A_3 receptor with a K_i value of 15 nM (A_{2A}/A_3 selectivity ratio of 4). In general, compounds with a relatively small 5′-*S*-alkyl-5′-thio substituent (methyl-5′-thio) displayed the highest affinities for both the adenosine A_{2A} and A_3 receptor; the larger ones (*n*- or *i*-propyl-5′-thio) increased the selectivity for the adenosine A_3 receptor. The novel compounds were also evaluated in cAMP assays for their (partial) agonistic behavior. Overall, the disubstituted derivatives behaved as partial agonists for both the adenosine A_{2A} and A_3 receptor. The compounds showed somewhat higher intrinsic activities on the adenosine A_{2A} receptor than on the A_3 receptor. Compounds **37**, **40** and **45**, **48**, with either a 5′-*S*-methyl-5′-thio or a 5′-*S*-*i*-propyl-5′-thio substituent had the lowest intrinsic activities on the adenosine A_{2A} receptor. For the A_3 receptor, compounds **34**, **35**, **38**, **39,** and **46**, **47**, with a 5′-*S*-ethyl-5′-thio or a 5′-*S*-*n*-propyl-5′-thio substituent had the lowest intrinsic activities.

Introduction

The endogenous neuromodulator adenosine acts extracellularly via activation of specific membrane-bound receptors called P_1-purinoceptors. These adenosine receptors can be divided into four subclasses, A_1, A_{2A}, A_{2B}, and A_3 receptors. All four classes are coupled to the enzyme adenylate cyclase. Activation of the adenosine A_1 and A_3 receptors leads to an inhibition of adenylate cyclase, while activated A_{2A} and A_{2B} receptors stimulate adenylate cyclase. The adenosine receptors are ubiquitously distributed throughout the body. As a consequence, ligands need to be highly selective in their action with respect to receptor subtype and tissue to be of therapeutic value.

Receptor subtype selectivity can be achieved by substituting the adenosine molecule. For example, modification at the N^6-position of adenosine is well tolerated. N^6-Substituents such as cyclopentyl enhance adenosine A_1 receptor selectivity relative to the other subtypes,[1,2] while a 3-iodobenzyl group induces adenosine A_3 receptor selectivity.[3−5] Bulky substituents such as (ar)alkylamino,[6] alkylidenehydrazino,[7] and alkynyl[8] at the 2-position of the adenine moiety have been described to yield selectivity for the adenosine A_{2A}

receptor compared to A_1. Only more recently, the 2-(ar)-alkynyl adenosine derivatives have been evaluated at the adenosine A_3 receptor. Quite surprisingly, some of these compounds appeared to be selective for the adenosine A_3 receptor rather than for A_{2A}.[9,10]
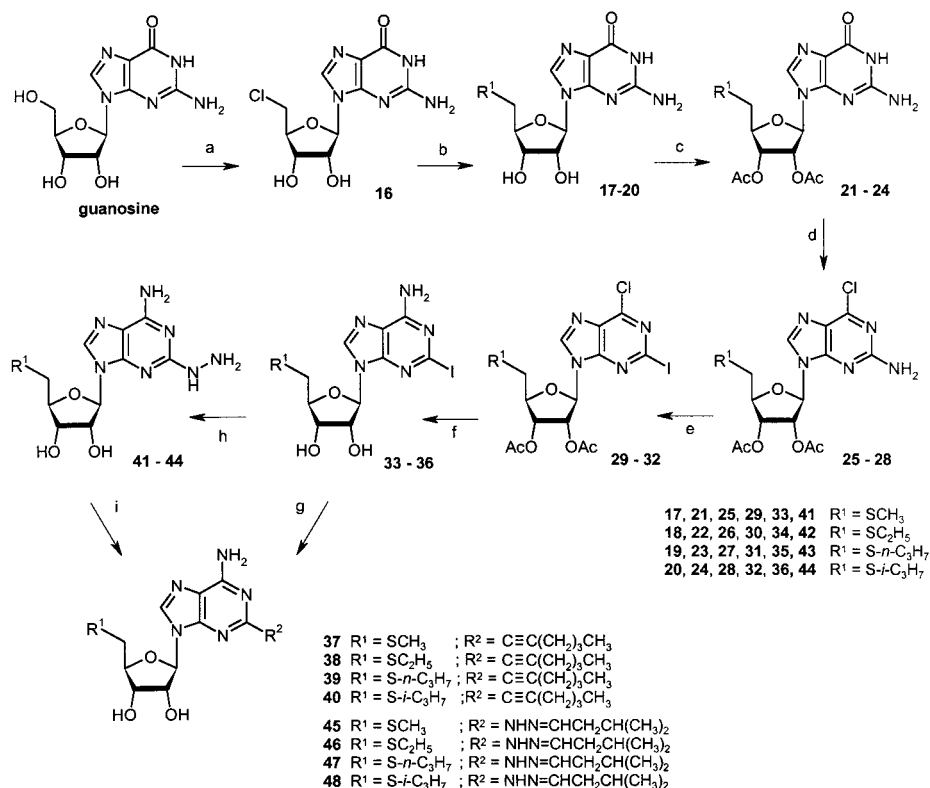
Tissue selectivity is often the result of partial agonism, which may reduce the extent of side effects.[11,12] Due to differences in receptor−effector coupling in various tissues, selectivity of action in vivo may be achieved. Partial agonists for the adenosine A_{2A} receptor may be of use as antipsychotic drugs, since stimulation of the A_{2A} receptor leads to inhibition of dopamine D_2 receptor activation in the basal ganglia.[13,14] Partial adenosine A_3 agonists can act as cardio- and cerebroprotective agents after chronic administration.[15,16] Hopefully, with partial agonists the significant decrease in blood pressure caused by vasodilation (A_{2A}) and bronchoconstriction in the lung and the release of allergic mediators in the immune system (A_3) may be avoided.[17]

In the present study the synthesis and biological evaluation are described for a series of disubstituted adenosine analogues. At the 2-position these derivatives contain either a 2-(1-hexynyl) or a 2-(*N*-3-methyl-1-butylidenehydrazino) group, to assess their effect on selectivity. At the 5′-position, alkylthio groups were introduced for partial agonism, since recent studies from our laboratory have shown that these substituents led to a reduced efficacy at both the adenosine A_1 and A_3 receptor.[1,5] Hence, compounds with both a 2- and 5′-

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Scheme 1. Synthesis of 2-Substituted Adenosine Derivatives[a]
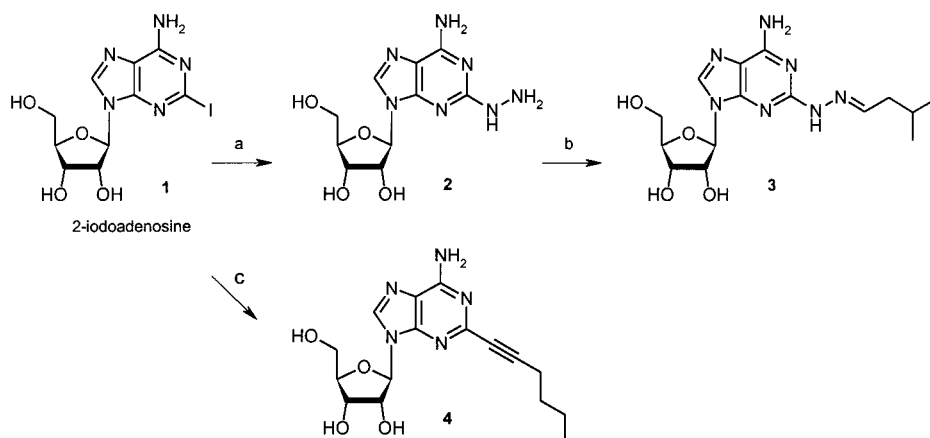
[a] Reagents and conditions: (a) 2-propanol, hydrazine monohydrate, reflux; (b) MeOH, isovalderaldehyde, reflux; (c) CH$_3$CN, Et$_3$N, CuI, PdCl$_2$, Ph$_3$P, 1-hexyn.

Scheme 2. Synthesis of 2,5′-Disubstituted Adenosine Derivatives[a]

17, 21, 25, 29, 33, 41   R^1 = SCH$_3$
18, 22, 26, 30, 34, 42   R^1 = SC$_2$H$_5$
19, 23, 27, 31, 35, 43   R^1 = S-n-C$_3$H$_7$
20, 24, 28, 32, 36, 44   R^1 = S-i-C$_3$H$_7$

37 R^1 = SCH$_3$; R^2 = C≡C(CH$_2$)$_3$CH$_3$
38 R^1 = SC$_2$H$_5$; R^2 = C≡C(CH$_2$)$_3$CH$_3$
39 R^1 = S-n-C$_3$H$_7$; R^2 = C≡C(CH$_2$)$_3$CH$_3$
40 R^1 = S-i-C$_3$H$_7$; R^2 = C≡C(CH$_2$)$_3$CH$_3$

45 R^1 = SCH$_3$; R^2 = NHN=CHCH$_2$CH(CH$_3$)$_2$
46 R^1 = SC$_2$H$_5$; R^2 = NHN=CHCH$_2$CH(CH$_3$)$_2$
47 R^1 = S-n-C$_3$H$_7$; R^2 = NHN=CHCH$_2$CH(CH$_3$)$_2$
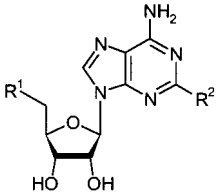48 R^1 = S-i-C$_3$H$_7$; R^2 = NHN=CHCH$_2$CH(CH$_3$)$_2$

[a] Reagents and conditions: (a) HMPA, SOCl$_2$, Dowex 50 W (H$^+$); (b) (i) RSH, 2 M NaOH, reflux, (ii) acetic acid; (c) (i) DMAP, CH$_3$CN, Et$_3$N, acetic anhydride, (ii) MeOH; (d) CH$_3$CN, Et$_4$NCl, N,N-dimethylaniline, POCl$_3$, reflux; (e) I$_2$, CH$_2$I$_2$, CuI, isopentylnitrite, THF; (f) EtOH/NH$_3$; (g) CH$_3$CN, Et$_3$N, CuI, PdCl$_2$, Ph$_3$P, 1-hexyn; (h) 2-propanol, hydrazine monohydrate, reflux; (i) MeOH, isovalderaldehyde, reflux.

substituent were synthesized, and their affinities were determined with radioligand binding assays. Their intrinsic activities were determined in cAMP assays.

Chemistry

The synthesis routes to obtain the 2-substituted adenosine derivatives **2**−**4** and the 2,5′-disubstituted derivatives **33**−**40** and **45**−**48** are depicted in Schemes 1 and 2, respectively. The synthesis of compounds **2**−**4** started with 2-iodoadenosine (**1**), which is an important intermediate for the synthesis of several adenosine derivatives, described in the literature.[18] 2-Hydrazinoadenosine (**2**) was synthesized starting from **1** by

stirring it with hydrazine monohydrate at 80 °C. 2-(N-3-Methyl-1-butylidenehydrazino)-adenosine (**3**) was generated by the condensation of **2** with isovaleraldehyde according to the method of Niiya et al.[7] 2-(1-Hexynyl)-adenosine (**4**) was prepared in good yield (88%) by reacting **1** with 1-hexyn.[8,19] Part of the synthesis route for compounds **33**−**40** and **45**−**48** was similar to that of compounds **2**−**4**, except for the fact that prior to C-2 substitution, the 5′-S-alkyl-5′-thio substituents were introduced. In fact, this was performed in an early stage of the route, i.e., at commercially available guanosine, which was also used as the starting material for 2-iodoadenosine (**1**) itself. The 5′-S-alkyl-5′-thio-substi-

Table 1. Affinities of 2,5′-Disubstituted Adenosine Analogues at Adenosine A_1, A_{2A}, and A_3 Receptors Expressed as K_i Values ($\pm$ SEM in nM, $n = 3$) or Percentage Displacement at 10 μM

| no. | R^1 | R^2 | K_i (nM) or % displacement at 10^{-5} M | | |
			$A_1{}^a$	$A_{2A}{}^b$	$A_3{}^c$
	CGS21680[41]		2600^d	15^e	584^f
	NECA[32]		63	16	11
	Cl-IB-MECA		710 ± 41	23.5%	7.2 ± 0.9
1	OH	I	36.1%	4200 ± 80	297 ± 17
33	SCH_3	I	42.8%	3900 ± 580	257 ± 35
34	SC_2H_5	I	386 ± 384	1200 ± 120	395 ± 61
35	S-n-C_3H_7	I	1050 ± 485	440 ± 50	558 ± 179
36	S-i-C_3H_7	I	56.2%	820 ± 180	546 ± 86
4	OH	$C{\equiv}C(CH_2)_3CH_3$	63.7%	6 ± 1	16.9 ± 4.1
37	SCH_3	$C{\equiv}C(CH_2)_3CH_3$	35.9%	60 ± 20	14.5 ± 3.4
38	SC_2H_5	$C{\equiv}C(CH_2)_3CH_3$	2180 ± 1980	110 ± 30	32.3 ± 11.8
39	S-n-C_3H_7	$C{\equiv}C(CH_2)_3CH_3$	46.5%	170 ± 10	88.3 ± 6.6
40	S-i-C_3H_7	$C{\equiv}C(CH_2)_3CH_3$	1270 ± 740	220 ± 10	75.4 ± 43.1
3	OH	$NHN{=}CHCH_2CH(CH_3)_2$	18.9%	20 ± 7	38.3 ± 3.3
45	SCH_3	$NHN{=}CHCH_2CH(CH_3)_2$	18.9%	220 ± 20	253 ± 36
46	SC_2H_5	$NHN{=}CHCH_2CH(CH_3)_2$	21.2%	500 ± 40	814 ± 132
47	S-n-C_3H_7	$NHN{=}CHCH_2CH(CH_3)_2$	22.0%	1500 ± 280	697 ± 31
48	S-i-C_3H_7	$NHN{=}CHCH_2CH(CH_3)_2$	13.0%	1800 ± 230	409 ± 118

^{a} Displacement of [^{3}H]DPCPX from rat cortical membranes.[36] ^{b} Displacement of [^{3}H]ZM241385 from rat striatal membranes.[37] ^{c} Displacement of [^{125}I]AB MECA from the human A_3 receptor expressed in HEK 293 cells.[4,40] ^{d} Displacement of [^{3}H]CHA binding from rat cortical membranes.[41] ^{e} Displacement of [^{3}H]NECA binding from rat striatal membranes.[41] ^{f} Displacement of [^{125}I]APNEA.[41]

tuted derivatives **17**−**20** were obtained by reacting 5′-chloro-5′-deoxy-guanosine (**16**) with the appropriate thiol in 2 M NaOH.[1,5,20] Subsequently, the 2′,3′-hydroxyl groups were acetyl protected to avoid complications during further synthesis, and compounds **21**−**24** were obtained in quantitative yields.

Chlorination on the 6-position of compounds **21**−**24** was performed with phosphoryl chloride (POCl$_3$),[18,21] yielding 2-amino-6-chloro-9-(2,3-di-O-acetyl-5-S-alkyl-5-thio-β-D-ribo-furanosyl)-purine derivatives **25**−**28** in reasonable to good yields (40−74%). Subsequently, the 2-amino group of compounds **25**−**28** was replaced by iodine via a diazotization-iodine substitution reaction.[22] This method appeared to be an efficient way to prepare **29**−**32**. The 5′-S-alkyl-2-iodo-5′-thioadenosine intermediates, **33**−**36**, were obtained by stirring **29**−**32** with ethanol saturated with ammonia. The acetyl protecting groups were readily removed under these conditions. The 6-position, however, was aminated only after a few days. Finally, as for compound **3**,[8] the 2-(1-hexynyl) group could be easily introduced in **33**−**36**, and the 5′-S-alkyl-2-(1-hexynyl)-5′-thioadenosine derivatives **37**−**40** were obtained in good yields. The 5′-S-alkyl-2-(N-3-methyl-1-butylidenehydrazino)-5′-thioadenosine derivatives (**45**−**48**) were synthesized by the condensation of the 5′-S-alkyl-2-hydrazino-5′-thioadenosine derivatives (**41**−**44**) with isovaleraldehyde as described above.[7]

Biological Evaluation

All compounds were tested in radioligand binding assays to determine their affinities for the adenosine A_1 receptor in rat brain cortex, the A_{2A} receptor in rat striatum, and the human A_3 receptor as expressed in HEK 293 cells (Table 1). For the adenosine A_1 receptor,

the tritiated antagonist [^{3}H]-1,3-dipropyl-8-cyclopentylxanthine ([^{3}H]DPCPX) and, for the adenosine A_{2A} receptor, the tritiated antagonist [^{3}H]ZM241385 (7-amino-2-(2-furyl)-5-[2-(4-hydroxyphenyl)ethyl]amino-[1,2,4]-triazolo[1,5-a][1,3,5]triazine) were used. Since radio-labeled antagonists are not commercially available for the adenosine A_3 receptor, [^{125}I] AB-MECA (N^6-(4-aminobenzyl)-5′-methylcarboxamidoadenosine), an A_3 receptor agonist, was used. Displacement experiments were performed in the absence of GTP.

All compounds were also tested in functional assays. The ability of the compounds (**1**, **3**, **4**, **33**−**40**, and **45**−**48**) to either stimulate the cyclic AMP (cAMP) production through human adenosine A_{2A} receptors expressed in CHO cells or inhibit the cAMP production in human adenosine A_3 receptors expressed in HEK 293 cells was assessed.

Results and Discussion

The synthesis of compounds **2**−**4** started with 2-iodoadenosine (**1**, Scheme 1).[18] Substitution of the 2-iodo group of compound **1** with the desired substituents was quite straightforward. The 2-(N-3-methyl-1-butylidenehydrazino) derivative (**3**) was synthesized by the condensation of 2-hydrazinoadenosine (**2**) with isovaleraldehyde.[7] The complete conversion of the iodonucleoside to its 2-(1-hexynyl) derivative (**4**) was carried out by a modification of the traditional palladium-catalyzed cross-coupling reaction.[8]

The synthesis of compounds **33**−**40** and **45**−**48** appeared to be more difficult. The 2-substituent should preferably be introduced prior to the 5′-substituent, since the latter was mostly varied. However, introduction of a good leaving group at the 5′-position of

compounds **2**−**4** failed. The use of the chlorination method described by Robins et al.[5,20,23] either decomposed the starting material (**2**) or resulted in the addition of chlorine to the multiple bond of the 2-substituent (**3** or **4**), besides 5′-chlorination. Milder chlorination conditions, such as the use of carbon tetrahalides and triphenylphosphine,[24,25] or straightforward mild Mitsunobu reaction conditions, however, regained only starting materials (with the 2′- and 3′-hydroxyl groups either protected or unprotected).

These results showed us that it proved to be very difficult to introduce the 2-substituent prior to the 5′-substituent. In Scheme 2, the successful synthesis of the desired compounds is depicted. Although more laborious, this route starts with the introduction of the 5′-*S*-alkyl-5′-thio substituents already at guanosine to circumvent all problems. Chlorination[20] of guanosine gave 5′-chloro-5′-deoxy-guanosine (**16**) in 93% yield. Compound **16** was reacted with the appropriate thiol in 2 M NaOH,[1,5] and the 2′- and 3′-hydroxyl groups of compounds **17**−**20** were subsequently protected to yield the 2′,3′-*O*-acetyl-5′-*S*-alkyl-5′-thio-substituted derivatives **21**−**24**. Several laboratories have described chlorination on the 6-position of purine nucleosides with phosphoryl chloride (POCl$_3$).[18,21] To prevent decomposition of the starting materials, the chemicals used were either properly dried or freshly distilled, and compounds **25**−**28** were obtained in reasonable to good yields (40%−74%). Subsequently, the 2-amino group of compounds **25**−**28** was replaced by iodine. The method used[26] is a variation of the originally diazotization−iodine substitution procedure.[22] With this efficient method **29**−**32** were obtained in good yields (71−82%). Stirring **29**−**32** in ethanol freshly saturated with NH$_3$ readily removed the protecting groups and slowly aminated the 6-position to give the intermediates **33**−**36**. NMR showed that amination at the 6-position needed stirring at room temperature for at least 2 days. Subsequent introduction of the 2-substituents was done as described above,[7,8] and compounds **37**−**40** and **45**−**48** were obtained in good yields.

Table 1 displays radioligand-binding data for all synthesized final products **1**, **3**, **4**, **33**−**40**, and **45**−**48**. From this table it is clear that most compounds had very low or negligible affinity for the adenosine A$_1$ receptor. Also, most compounds did not show any preference in binding at either the adenosine A$_{2A}$ or the A$_3$ receptor, with reasonable to good affinities for both receptor subtypes. Some compounds were slightly selective for the adenosine A$_{2A}$ receptor (**3**, **4**) with an A$_{2A}$/A$_3$ selectivity ratio significantly smaller than unity, while other compounds favored the adenosine A$_3$ receptor more (**1**, **33**−**34**, **38**, **48**) with an A$_{2A}$/A$_3$ selectivity ratio up to 15-fold (**33**). These results corroborate data found in the literature. Substitution at the 2-position is known to increase selectivity for the adenosine A$_{2A}$ receptor.[7,8] More recently, radioligand binding studies of 2-(ar)-alkynyl-substituted adenosine derivatives have shown that, besides A$_1$/A$_{2A}$ selectivity, also A$_1$/A$_3$ selectivity is increased by these 2-substituents.[9,10,27] Indeed, the 2-(1-hexynyl) substituent induced high affinity for both the adenosine A$_{2A}$ and A$_3$ receptor compared to the A$_1$ receptor. High affinities for both the adenosine A$_{2A}$ and the A$_3$ receptor were also obtained with the 2-(*N*-3-

methylbutylidenhydrazino) derivatives, which had previously been tested in functional assays for the adenosine A$_1$ and A$_{2A}$ receptor only.[7] Clearly, 2-substitution does not merely induce adenosine A$_{2A}$ receptor selectivity as commonly accepted in the literature, as for many 2-substituted adenosine derivatives A$_3$ receptor affinities are not reported. The 2-(1-hexynyl) derivatives (**4**, **37**−**40**) had the highest affinities for both the adenosine A$_{2A}$ and A$_3$ receptors, compared to the 2-(*N*-3-methylbutylidene)hydrazino and 2-iodo substituted compounds. Compound **4** had the highest affinity for the adenosine A$_{2A}$ receptor (K_i value of 6 nM), while compound **37** had the highest affinity for the adenosine A$_3$ receptor (K_i value of 14.5 nM). The adenosine A$_{2A}$ receptor is known to accommodate only 2-substituents with a restrained spacer.[28] This explains the rather low affinities of the 2-iodo derivatives (**1**, **33**−**36**) for this receptor and the good affinities of the compounds with larger 2-substituents (1-hexynyl or *N*-3-methylbutylidenehydrazino) that contain a relatively rigid spacer. The affinities of the 2-iodo derivatives for the adenosine A$_3$ receptor are significantly better than for the A$_{2A}$ receptor, indicating that the C2 region of the adenosine A$_3$ receptor might be less restrained.

Few substituted adenosine derivatives have been tested recently on the human adenosine A$_{2A}$ instead of the rat A$_{2A}$ receptor. Compounds such as CPA, CGS21680, NECA, DMPA (*N*^{6}-[2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl)ethyl]adenosine), and HENECA display lower affinity for the human adenosine A$_{2A}$ receptor than for the rat A$_{2A}$ receptor.[29−31] These data suggest that our synthesized compounds might also have lower affinities for the human adenosine A$_{2A}$ receptor and therefore may be even more selective for the adenosine A$_3$ receptor.

Although the 5′-substituents were primarily introduced to induce partial agonism, they also had an effect on the affinity of the compounds. A similar change in affinity was observed for both the adenosine A$_{2A}$ and the A$_3$ receptor. On most occasions, a bulky 5′-substituent led to a decrease in affinity for both receptor subtypes. Only in the 5′-substituted 2-iodoadenosine series (**1**, **33**−**36**) adenosine A$_{2A}$ receptor affinity seemed to increase with a bulky substituent. The 5′-hydroxyl derivatives **3** and **4** had high affinities for both the adenosine A$_{2A}$ and the A$_3$ receptor. The general trend within all three series is that the A$_{2A}$ and A$_3$ receptor affinity decreases with increasing size of the 5′-substituent (except for the A$_{2A}$ receptor affinities of the 2-iodo substituted derivatives). Within the 2-iodoadenosine series, increasing size of the 5′-substituent led to a 10-fold increase in A$_3$/A$_{2A}$ selectivity. On the other hand, within the 2-(1-hexynyl) and 2-(*N*-3-methylbutylidenehydrazino) series, the larger 5′-substituents increased adenosine A$_3$ selectivity, with a 9- and 10-fold decrease of A$_3$/A$_{2A}$ selectivity, respectively. This is in line with our previously reported data on *N*^{6},5′-disubstituted adenosine derivatives.[5] There too, the introduction of 5′-*S*-alkyl-5′-thio substituents at adenosine derivatives increased the selectivity for the adenosine A$_3$ receptor compared to the A$_{2A}$ receptor. The question remains how this change in selectivity induced by the 5′-*S*-alkyl-5′-thio substituents can be understood. Steric effects cannot be the explanation for the decrease

Table 2. EC$_{50}$ Values and Maximum Levels of G Protein Activation (E_{max}) of 2,5′-Disubstituted Adenosine Derivatives at the A$_{2A}$ Receptor and the E_{max} Values at the A$_3$ Receptor as Determined in cAMP Assays
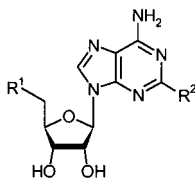
no.	R^1	R^2	E_{max} (%) A$_{2A}$[a]	EC$_{50}$ (μM) CHO cells A$_{2A}$	E_{max} (%) A$_3$[b]
	CGS21680		100		
	NECA		102 ± 23	0.04 ± 0.004	
	Cl-IB-MECA				83 ± 2 (10)
1	OH	I	112 ± 7	5.4 ± 0.7	68 ± 7 (30)
33	SCH$_3$	I	n.a.[c]		41 ± 17 (30)
34	SC$_2$H$_5$	I	n.a.[c]		14 ± 10 (30)
35	S-n-C$_3$H$_7$	I	n.a.[c]		10 ± 6 (30)
36	S-i-C$_3$H$_7$	I	n.a.[c]		17 ± 13 (30)
4	OH	C≡C(CH$_2$)$_3$CH$_3$	105 ± 4	0.010 ± 0.0003	79 ± 8 (3)
37	SCH$_3$	C≡C(CH$_2$)$_3$CH$_3$	45 ± 6	0.7 ± 0.1	72 ± 9 (3)
38	SC$_2$H$_5$	C≡C(CH$_2$)$_3$CH$_3$	79 ± 10	0.5 ± 0.02	50 ± 21 (3)
39	S-n-C$_3$H$_7$	C≡C(CH$_2$)$_3$CH$_3$	82 ± 7	0.8 ± 0.1	33 ± 29 (10)
40	S-i-C$_3$H$_7$	C≡C(CH$_2$)$_3$CH$_3$	32 ± 4	3.6 ± 1.5	49 ± 22 (10)
3	OH	NHN=CHCH$_2$CH(CH$_3$)$_2$	92 ± 2	1.1 ± 0.2	24 ± 17 (3)
45	SCH$_3$	NHN=CHCH$_2$CH(CH$_3$)$_2$	44 ± 4	5.0 ± 0.6	41 ± 16 (30)
46	SC$_2$H$_5$	NHN=CHCH$_2$CH(CH$_3$)$_2$	101 ± 4	7.5 ± 0.3	7 ± 37 (100)
47	S-n-C$_3$H$_7$	NHN=CHCH$_2$CH(CH$_3$)$_2$	126 ± 3	12.9 ± 0.9	16 ± 14 (100)
48	S-i-C$_3$H$_7$	NHN=CHCH$_2$CH(CH$_3$)$_2$	56 ± 1	10.4 ± 1.0	26 ± 8 (30)

[a] E_{max} compared to the E_{max} of CGS 21680 (± SEM, $n = 3$; 10 μM) in A$_{2A}$ CHO cells. [b] Percentage of inhibition of forskolin-induced (10 μM) cAMP production, compared to Cl-IB-MECA. In parentheses: the concentration at which the effect was determined (μM, approximately 100 × K_i value). [c] Not active (no cAMP produced at 30 μM (**35**) or 100 μM; approximately 100 × K_i value).

in adenosine A$_{2A}$ receptor affinity, since the size of, in particular, the 5′-S-ethyl-5′-thio group fairly matches that of a 5′-N-ethylcarboxamido substituent, as in NECA and CGS21680. While MECA is known to have a higher affinity for the adenosine A$_3$ receptor than NECA,[32] our results suggest that the adenosine A$_3$ receptor is better able to accommodate the larger 5′-S-alkyl-5′-thio groups than the A$_{2A}$ receptor. In literature, modifications other than 5′-S-alkyl-5′-thio and 5′-N-alkylcarboxamido have been investigated for their affinities for the adenosine A$_{2A}$ or A$_3$ receptor. Mogensen et al. have shown that the relatively large 3-isoxazolyl substituent at the 5′-position of adenosine derivatives indeed decreases adenosine A$_{2A}$ receptor affinity compared to its 5′-N-methylcarboxamido derivative.[33] On the contrary, for polysubstituted adenosine derivatives with a 5-isoxazolyl substituent, it has been claimed that these compounds have a very high adenosine A$_{2A}$ receptor affinity and that they are selective compared to A$_3$.[34]

Table 2 shows the effects of the synthesized compounds in cAMP assays. All compounds were first tested at a final single concentration of 1−100 μM (25 to 166 times the K_i value), for determination of the amount of cAMP produced via the adenosine A$_{2A}$ receptor. For compounds **1**, **4**, **37−40**, **3**, and **45−48**, full dose−response curves were recorded next. The E_{max} values were determined from the fitted curves and compared to the maximal amount of cAMP (E_{max}) produced by the reference full agonist, CGS21680 (10 μM).

Surprisingly, the 5′-substituted 2-iodo derivatives **33−36** did not produce any cAMP. This suggested either that they behaved as antagonists in this assay, or that they were substrates for adenosine deaminase (ADA) present in the assay. The latter explanation is however

unlikely, since these compounds did not seem to be substrates for ADA in the cAMP assay for the adenosine A$_3$ receptor, in which ADA was present as well. All adenosine derivatives (**1**, **3**, and **4**) produced similar amounts of cAMP as the full agonist CGS21680, suggesting that they behaved as full agonists. The 5′-substituted derivatives within the 2-(1-hexyn-1-yl) series (Table 2) were partial agonists compared to **4** and CGS 21680. The 5′-substituted 2-(N-3-methylbutyl-idenehydrazino) derivatives showed a similar trend in cAMP production, although with higher efficacies. The efficacies of compounds **46** and **47** were similar to that of the full agonist CGS21680, and thus **46** and **47** behaved as full agonists for this receptor. Overall, most of the 2,5′-disubstituted adenosine derivatives behaved as partial agonists for the adenosine A$_{2A}$ receptor in this assay. The compounds with either a 5′-S-ethyl-5′-thio or a 5′-S-i-propyl-5′-thio substituent (**37**, **40**, **45,** and **48**) had the lowest efficacies (Table 2). The EC$_{50}$ values for the adenosine A$_{2A}$ receptor were up to 54-fold higher than the K_i values. The rank orders of potency and affinity were identical, however. It must be noted that the EC$_{50}$ values were determined for the human adenosine A$_{2A}$ receptor expressed in CHO cells, whereas the K_i values were determined on rat striatum.[35]

The ability of the compounds to inhibit forskolin-stimulated (10 μM) cAMP production via the adenosine A$_3$ receptor was also studied. All compounds were tested at a single (final) concentration of 3−100 μM (73 to 200 times the K_i value). The 2-iodo substituted derivatives (**1**, **33−36**) inhibited the forskolin-induced cAMP production via the adenosine A$_3$ receptor, proof of the agonistic character of the compounds that were silent on the adenosine A$_{2A}$ receptor. Compounds **1** and **4**, with an intact 5′-hydroxyl group, showed almost full inhibi-

tion of the cAMP production comparable with the reference full agonist Cl-IB-MECA (2-chloro-N^6-(3-iodobenzyl)-5′-methylcarboxamidoadenosine). Compound **3** only gave 24% inhibition of the cAMP production, indicating that it behaved as a partial agonist for the adenosine A_3 receptor. The 5′-substituted derivatives all showed submaximal levels of inhibition of the forskolin-induced cAMP production, with the same trend within the three 2-substituted series (Table 2). This indicates that the disubstituted adenosine derivatives were all partial agonists for the adenosine A_3 receptor in this assay. The compounds with the 5′-S-methyl-5′-thio substituent (**33**, **37**, **45**) had the highest intrinsic efficacies, whereas the compounds with a relatively large 5′-substituent (S-n-propyl-5′-thio, **35** and **39**) had the lowest intrinsic activities for the adenosine A_3 receptor. The partial agonistic behavior of 5′-S-alkyl-5′-thio-substituted adenosine derivatives is in line with data reported previously by our laboratory, where effects of 5′-substituents such as alkylseleno or alkylthio on the intrinsic activity of adenosine analogues were investigated. It was shown that the intrinsic activity of these compounds was reduced at both the adenosine A_1 and A_3 receptor,[1,5] leading to partial agonism for these receptors, respectively.

Conclusions

The 2,5′-disubstituted adenosine derivatives described in the present study were synthesized in good overall yields, starting from commercially available guanosine. It appeared that the compounds containing either a 2-(1-hexynyl) or a 2-(N-3-methyl-1-butylidene)hydrazino substituent were not merely adenosine A_{2A} receptor selective. This study provided evidence that these compounds also have good affinity for the adenosine A_3 receptor. The question remains whether compounds with 2-substituents other than those described here will be truly adenosine A_{2A} receptor selective, since binding data for such compounds on the adenosine A_3 receptor is often lacking. Ligands with high affinities were synthesized, although selectivities were at best modest. Compound **4** had the highest affinity for the adenosine A_{2A} receptor with a K_i value of 6 nM, whereas compound **37** had the highest affinity for the adenosine A_3 receptor with a K_i value of 15 nM. The 5′-substituent also had an effect on affinity for both the adenosine A_{2A} and A_3 receptor. Increasing the size of the 5′-substituent led to a decrease in affinity for both receptors. In general, the affinity for the adenosine A_{2A} receptor was decreased more significantly than that for the A_3 receptor, and A_3 selectivity was increased. The 5′-substituents also induced partial agonism for both the adenosine A_{2A} and A_3 receptor, and except for compounds **46** and **47** for the A_{2A} receptor, all 2,5′-disubstituted adenosine derivatives behaved as partial agonists for both receptor subtypes. In general, the synthesized compounds have lower intrinsic activities for the adenosine A_3 receptor than for the A_{2A} receptor. Surprisingly, compound **3** with an intact 5′-hydroxyl group showed partial agonism for the adenosine A_3 receptor as well. Partial agonists are useful pharmacological tools. Furthermore, partial agonists for the adenosine A_{2A} and A_3 receptor may have interesting new therapeutic potential as drugs, such as

antipsychotics or as cardio- and cerebroprotective agents, respectively, with potential reduction of side effects.

Experimental Section

Chemicals and Solvents. Guanosine was obtained from Aldrich (Aldrich Chemie, Sigma-Aldrich Chemie BV, Zwijndrecht, The Netherlands). All other reagents were from standard commercial sources and of analytic grade. [³H]-DPCPX (1,3-dipropyl-8-cyclopentylxanthine), [³H]ZM241385, and [¹²⁵I]AB-MECA were purchased from NEN (Hoofddorp, The Netherlands).

Chromatography. Thin-layer chromatography (TLC) was carried out using aluminum sheets (20 × 20 cm) with silica gel F_{254} from Merck. Spots were visualized under UV (254 nm). Preparative column chromatography was performed on silica gel (230−400 mesh ASTM).

Instruments and Analyses. Elemental analyses were performed for C, H, N (Department of Analytical Chemistry, Leiden University, The Netherlands). ¹³C NMR spectra were measured at 50.1 MHz with a JEOL JNM-FX 200 spectrometer equipped with a PG 200 computer operating in the Fourier transform mode. ¹H NMR spectra were measured at 200 MHz, using the above-mentioned spectrometer, or at 300 MHz, using a Bruker WM-300 spectrometer equipped with an ASPECT-2000 computer operating in the Fourier transform mode. Chemical shifts for ¹H and ¹³C NMR are given in ppm (δ) relative to tetramethylsilane (TMS) as internal standard.

All high-resolution mass spectra were measured on a Finnigan MAT900 mass spectrometer equipped with a direct insertion probe for EI experiments (70 eV with resolution 1000) or on a Finnigan MAT TSQ-70 spectrometer equipped with an electrospray interface for ESI experiments. Spectra were collected by constant infusion of the analyte dissolved in 80/20 methanol/H_2O. ESI is a soft ionization technique resulting in protonated, sodiated species in positive ionization mode and deprotonated species in the negative ionization mode.

Resolution of the compounds was achieved by reverse-phase HPLC (Gilson HPLC system, 712 system controller software, Gilson Netherlands, Meyvis en Co BV, Bergen op Zoom, The Netherlands) using a 65% MeOH/31.5% H_2O/3.5% CH_3CN (v/v) mobile phase and an Alltima C18 5 μm (250 mm × 4.6 mm) or a nucleotide/nucleoside 7 μm (250 mm × 4.6 mm) column (Alltech Nederland BV, Breda, The Netherlands) at a flow rate of 0.7 mL/min. The peaks were defined by measurement of UV absorbance (254 nm). Retention times are given.

Melting points (not corrected) were determined in a Büchi capillary melting point apparatus.

Syntheses. 5′-Chloro-5′-deoxyguanosine (16). Guanosine (43.5 g, 0.15 mol) was dissolved in hexamethylphosphorictriamide (HMPA, 40 mL, 0.23 mol). Thionyl chloride (61.5 mL, 0.85 mol) was added in 1 h. The mixture was stirred at ambient temperature for 1 h, diluted with water, and chromatographed on Dowex 50 W (H⁺). After washing with water (350 mL), the product was collected by eluting 5% aqueous ammonia (350 mL). The fraction was concentrated in vacuo. Yield 40 g (0.13 mol, 86%), ¹H NMR (DMSO-d_6) δ 10.53 (bs, 1H, NH), 7.89 (s, 1H, H-8), 6.50 (bs, 2H, NH_2), 5.72 (d, J = 5.84 Hz, 1H, H-1′), 5.55 (d, J = 6.52 Hz, 1H, OH-2′), 5.39−5.35 (m, 1H, OH-3′), 4.57 (q, J = 5.15 Hz, 1H, H-2′), 4.16−4.05 (m, 1H, H-3′), 4.05−3.97 (m, 1H, H-4′), 3.86 (dq, J = 11.67 Hz, 2H, H-5′).

General Procedure for the Syntheses of 5′-S-Alkyl-5′-thio Derivatives 17−20. The appropriate thiol (3.32 mmol) was dissolved in 10 mL of 2 M NaOH. After the mixture was stirred, 5′-chloro-5′-deoxyguanosine (**16**; 100 mg, 0.33 mmol) was slowly added. The mixture was refluxed for 2−2.5 h and then cooled to room temperature. It was acidified with acetic acid, and a white precipitate was formed. The precipitate was filtered and dried.

5′-S-Methyl-5′-thioguanosine (17). The reaction was carried out with sodium thiomethoxide (27.42 g, 0.39 mol) and **16** (11.8 g, 39.1 mmol). Yield 10.41 g (33.2 mmol, 85%).

5′-*S*-Ethyl-5′-thioguanosine (18). The reaction was carried out with ethanethiol (20.6 mL, 0.28 mol) and **16** (8.40 g, 27.8 mmol). Yield 6.98 g (21.3 mmol, 77%).

5′-*S*-Propyl-5′-thioguanosine (19). The reaction was carried out with 1-propanethiol (27.0 mL, 0.30 mol) and **16** (9.0 g, 29.8 mmol). Yield 6.1 g (17.9 mmol, 60%).

5′-*S*-Isopropyl-5′-thioguanosine (20). The reaction was carried out with 2-propanethiol (30.8 mL, 0.33 mol) and **16** (10.0 g, 33.2 mmol). Yield 6.64 g (19.5 mmol, 59%).

General Acetylation Procedure of Derivatives 17−20 To Obtain 21−24. To a suspension of compound **17** (0.46 mmol) and 4-(dimethylamino)pyridine (DMAP; 0.03 mmol) in a mixture of acetonitrile (5.7 mL) and triethylamine (154 μL, 1.1 mmol) was added acetic anhydride (95 μL, 1 mmol) at room temperature. The mixture was stirred for 1 h until the solution became clear. Methanol (10 mL) was added, and the solution was stirred for 5−10 min, concentrated in vacuo, and stirred with 2-propanol. The white slurrie obtained was filtered and subsequently stirred with hexane. The white precipitate was filtered and dried.

2′,3′-Di-*O*-acetyl-5′-*S*-methyl-5′-thioguanosine (21). The reaction was carried out with 5′-*S*-methyl-5′-thioguanosine (**17**, 10.4 g, 33.2 mmol). Yield 10.5 g (26.4 mmol, 79%).

2′,3′-Di-*O*-acetyl-5′-*S*-ethyl-5′-thioguanosine (22). The reaction was carried out with 5′-*S*-ethyl-5′-thioguanosine (**18**, 6.98 g, 21.3 mmol). Yield 7.94 g (19.3 mmol, 91%).

2′,3′-Di-*O*-acetyl-5′-*S*-propyl-5′-thioguanosine (23). The reaction was carried out with 5′-*S*-propyl-5′-thioguanosine (**19**, 5.74 g, 16.8 mmol). Yield 5.71 g (13.4 mmol, 80%).

2′,3′-Di-*O*-acetyl-5′-*S*-isopropyl-5′-thioguanosine (24). The reaction was carried out with 5′-*S*-isopropyl-5′-thioguanosine (**20**, 6.64 g, 19.5 mmol). Yield 6.92 g (16.3 mmol, 83%).

General Chlorination Procedure of Derivatives 21−24 To Obtain 25−28. To a suspension of the appropriate 2′,3′-di-*O*-acetyl-5′-*S*-alkyl-5′-thioguanosine (19.3 mmol, predried) and tetraethylammonium chloride (6.48 g, 39.1 mmol; predried in vacuo at 80 °C) in acetonitrile (40 mL) were added *N,N*-dimethylaniline (2.52 mL, 20.0 mmol, dried and distilled from KOH) and phosphoryl chloride (POCl$_3$, 10.95 mL, 0.12 mol, freshly distilled) at room temperature. The flask was placed in an oil bath preheated at 100 °C, and the solution was refluxed for 10−15 min. Volatile materials were evaporated immediately in vacuo. The resulting yellow foam was dissolved in CH$_2$Cl$_2$ (100 mL) and stirred vigorously for 15 min with crushed ice. The layers were separated, and the aqueous phase was extracted with CH$_2$Cl$_2$ again (75 mL). The combined organic layers were kept cold by addition of crushed ice and washed with cold water (3 × 75 mL), 5% NaHCO$_3$/H$_2$O to pH 7, dried over MgSO$_4$, and filtered. The residue was purified by column chromatography.

2-Amino-6-chloro-9-(2,3-di-*O*-acetyl-5-*S*-methyl-5-thio-β-D-ribofuranosyl)-purine (25). The reaction was carried out with 2′,3′-di-*O*-acetyl-5′-*S*-methyl-5′-thioguanosine (**21**, 5.96 g, 15.0 mmol). The mixture was purified by column chromatography (eluent EtOAc:PE40/60 = 1:1 to 2:1). Yield 3.83 g (9.21 mmol, 62%), R_f 0.28 (EtOAc:PE40/60 = 2:1).

2-Amino-6-chloro-9-(2,3-di-*O*-acetyl-5-*S*-ethyl-5-thio-β-D-ribofuranosyl)-purine (26). The reaction was carried out with 2′,3′-di-*O*-acetyl-5′-*S*-ethyl-5′-thioguanosine (**22**, 7.94 g, 19.3 mmol). The mixture was purified by column chromatography (eluent EtOAc:PE40/60 = 3:2).

2-Amino-6-chloro-9-(2,3-di-*O*-acetyl-5-*S*-propyl-5-thio-β-D-ribofuranosyl)-purine (27). The reaction was carried out with 2′,3′-di-*O*-acetyl-5′-*S*-propyl-5′-thioguanosine (**23**, 5.71 g, 13.4 mmol). The mixture was purified by column chromatography (eluent EtOAc:PE40/60 = 1:1). Yield 4.41 g (9.91 mmol, 74%), R_f 0.44 (EtOAc:PE 40/60 = 2:1).

2-Amino-6-chloro-9-(2,3-di-*O*-acetyl-5-*S*-isopropyl-5-thio-β-D-ribofuranosyl)-purine (28). The reaction was carried out with 2′,3′-di-*O*-acetyl-5′-*S*-isopropyl-5′-thioguanosine (**24**, 6.72 g, 15.8 mmol). The mixture was purified by column chromatography (eluent EtOAc:PE40/60 = 1:1).

General Diazotization Method of Derivatives 25−28 To Obtain 29−32. Isopentylnitrite (23.2 mmol, 3.10 mL) was added to a mixture of the appropriate 2-amino-6-chloro-9-(2,3-di-*O*-acetyl-5-*S*-alkyl-5-thio-β-D-ribofuranosyl)-purine (7.49 mmol), I$_2$ (7.49 mmol, 1.90 g), CH$_2$I$_2$ (77.5 mmol, 6.24 mL), and CuI (7.87 mmol, 1.50 g) in 40 mL of tetrahydrofuran. The dark brown solution was refluxed (under intensive cooling) for 40−60 min and then cooled to room temperature. The mixture was filtered, and the filtrate was concentrated in vacuo. The residue was dissolved in CH$_2$Cl$_2$ and extracted with a saturated Na$_2$S$_2$O$_3$ solution, until the color disappeared. The organic layer was dried and concentrated. The brownish oil was purified by column chromatography.

6-Chloro-2-iodo-9-(2,3-di-*O*-acetyl-5-*S*-methyl-5-thio-β-D-ribofuranosyl)-purine (29). The reaction was carried out with 2-amino-6-chloro-9-(2,3-di-*O*-acetyl-5-*S*-methyl-5-thio-β-D-ribofuranosyl)-purine (**25**, 3.83 g, 9.21 mmol). The mixture was purified by column chromatography (eluent CH$_2$Cl$_2$−5% MeOH in CH$_2$Cl$_2$). Yield 3.99 g (7.58 mmol, 82%), R_f 0.62 (5% MeOH in CH$_2$Cl$_2$).

6-Chloro-2-iodo-9-(2,3-di-*O*-acetyl-5-*S*-ethyl-5-thio-β-D-ribofuranosyl)-purine (30). The reaction was carried out with 2-amino-6-chloro-9-(2,3-di-*O*-acetyl-5-*S*-ethyl-5-thio-β-D-ribofuranosyl)-purine (**26**, 3.22 g, 7.49 mmol). The mixture was purified by column chromatography (eluent CH$_2$Cl$_2$). Yield 2.88 g (5.33 mmol, 71%), R_f 0.71 (5% MeOH in CH$_2$Cl$_2$).

6-Chloro-2-iodo-9-(2,3-di-*O*-acetyl-5-*S*-propyl-5-thio-β-D-ribofuranosyl)-purine (31). The reaction was carried out with 2-amino-6-chloro-9-(2,3-di-*O*-acetyl-5-*S*-propyl-5-thio-β-D-ribofuranosyl)-purine (**27**, 4.41 g, 9.91 mmol). The mixture was purified by column chromatography (eluent EtOAc:PE40/60 = 1:1). Yield 4.02 g (7.23 mmol, 73%), R_f 0.50 (EtOAc:PE40/60 = 2:1).

6-Chloro-2-iodo-9-(2,3-di-*O*-acetyl-5-*S*-isopropyl-5-thio-β-D-ribofuranosyl)-purine (32). The reaction was carried out with 2-amino-6-chloro-9-(2,3-di-*O*-acetyl-5-*S*-isopropyl-5-thio-β-D-ribofuranosyl)-purine (**28**, 5.20 g, 11.7 mmol). The mixture was purified by column chromatography (eluent EtOAc:PE 40/60 = 1:1). Yield 4.88 g (8.76 mmol, 75%), R_f 0.47 (EtOAc:PE40/60 = 2:1).

General Procedure for Amination and Deprotection of Derivatives 29−32 To Obtain 33−36. The appropriate 6-chloro-2-iodo-9-(2,3-di-*O*-acetyl-5-*S*-alkyl-5-thio-β-D-ribofuranosyl)-purine (5.33 mmol) was stirred with 50 mL of EtOH/NH$_3$ for 64 h. The mixture was concentrated and purified by column chromatography.

2-Iodo-5′-*S*-methyl-5′-thioadenosine (33). The reaction was carried out with 6-chloro-2-iodo-9-(2,3-di-*O*-acetyl-5-S-methyl-5-thio-β-D-ribofuranosyl)-purine (**29**, 3.99 g, 7.58 mmol). The mixture was purified by column chromatography (10% MeOH in CH$_2$Cl$_2$). Yield 2.21 g (5.22 mmol, 69%), mp 90−93 °C; R_f 0.24 (10% MeOH in CH$_2$Cl$_2$). The product was recrystallized from EtOAc.

5′-*S*-Ethyl-2-iodo-5′-thioadenosine (34). The reaction was carried out with 6-chloro-2-iodo-9-(2,3-di-*O*-acetyl-5-ethyl-5-thio-β-D-ribofuranosyl)-purine (**30**, 2.88 g, 5.33 mmol). The mixture was purified by column chromatography (eluent EtOAc). Yield 2.05 g (4.69 mmol, 88%), mp 76−79 °C; R_f 0.15 (5% MeOH in CH$_2$Cl$_2$).

2-Iodo-5′-*S*-propyl-5′-thioadenosine (35). The reaction was carried out with 6-chloro-2-iodo-9-(2,3-di-*O*-acetyl-5-*S*-propyl-5-thio-β-D-ribofuranosyl)-purine (**31**, 4.02 g, 7.23 mmol). The mixture was purified by column chromatography (eluent 10% MeOH in CH$_2$Cl$_2$). Yield 2.35 g (5.21 mmol, 72%), mp 98−101 °C; R_f 0.30 (10% MeOH in CH$_2$Cl$_2$).

2-Iodo-5′-*S*-isopropyl-5′-thioadenosine (36). The reaction was carried out with 6-chloro-2-iodo-9-(2,3-di-*O*-acetyl-5-*S*-isopropyl-5-thio-β-D-ribofuranosyl)-purine (**32**, 4.88 g, 8.76 mmol). The mixture was purified by column chromatography (eluent 10% MeOH in CH$_2$Cl$_2$). Yield 2.77 g (6.13 mmol, 70%), mp 102−104 °C; R_f 0.33 (10% MeOH in CH$_2$Cl$_2$). The product was recrystallized from EtOAc.

General Procedure for the Introduction of a 1-Hexyn Group at Derivatives 1 and 33−36, To Obtain 4 and 37−40. To a solution of the appropriate 5′-*S*-alkyl-2-iodo-5′-thioadenosine (0.92 mmol) in 7 mL of dry acetonitrile and 7

mL of triethylamine under a nitrogen atmosphere was added CuI (0.07 mmol, 13.3 mg), PdCl$_2$ (0.05 mmol, 8.47 mg), and Ph$_3$P (0.11 mmol). To the suspension was added 1-hexyn (4.45 mmol, 511 μL), and the mixture was stirred overnight under nitrogen atmosphere. The light brown solution was filtered and concentrated. The residue was extracted with water and EtOAc (3 × 50 mL), the organic layer was dried, concentrated, and purified by column chromatography.

2-Iodoadenosine (1) was prepared according to literature.[18] Yield 80%; mp 185−187 °C; *R$_f$* 0.21 (10% MeOH in CH$_2$-Cl$_2$).

2-(1-Hexynyl)adenosine (4).[8] The reaction was carried out with 2-iodoadenosine (**1**, 440 mg, 1.12 mmol). The mixture was purified by column chromatography (eluent CH$_2$Cl$_2$ to 10% MeOH in CH$_2$Cl$_2$). Yield 330 mg (0.95 mmol, 85%), mp 106−109 °C; *R$_f$* 0.10 (10% MeOH in CH$_2$Cl$_2$). The product was recrystallized from CH$_2$Cl$_2$.

2-(1-Hexynyl)-5′-*S*-methyl-5′-thioadenosine (37). The reaction was carried out with 2-iodo-5′-*S*-methyl-5′-thioadenosine (**33**, 480 mg, 1.13 mmol). The mixture was purified by column chromatography (eluent CH$_2$Cl$_2$ to 10% MeOH in CH$_2$Cl$_2$). Yield 257 mg (0.68 mmol, 60%); mp 64−67 °C; *R$_f$* 0.28 (10% MeOH in CH$_2$Cl$_2$). The product was recrystallized from methanol; ^{1}H NMR (DMSO-*d$_6$*) δ 8.37 (s, 1H, H− 8), 7.39 (s, 2H, NH$_2$), 5.85 (d, *J* = 6.18 Hz, 1H, H-1′), 5.49 (d, *J* = 6.18 Hz, 1H, OH-2′), 5.32 (d, *J* = 4.81 Hz, 1H, OH-3′), 4.67 (q, *J* = 5.49 Hz, 1H, H-2′), 4.12−3.95 (m, 1H, H-3′), 4.12−3.95 (m, 1H, H-4′), 2.84 (t, *J* = 5.49 Hz, 2H, H-5′), 2.40 (t, *J* = 6.68 Hz, 2H, ≡CCH$_2$), 2.05 (s, 3H, SCH$_3$), 1.55−1.32 (m, 4H, ≡CCH$_2$C*H$_2$*C*H$_2$*), 0.90 (t, *J* = 6.18 Hz, 3H, CH$_3$); MS *m/z* 378 (M+H)$^+$; Anal. (C$_{17}$H$_{23}$N$_5$O$_3$S·0.56 CH$_3$OH) C, H, N.

5′-*S*-Ethyl-2-(1-hexynyl)-5′-thioadenosine (38). The reaction was carried out with 5′-*S*-ethyl-2-iodo-5′-thioadenosine (**34**, 400 mg, 0.92 mmol). The mixture was purified by column chromatography (eluent CH$_2$Cl$_2$ to 10% MeOH in CH$_2$Cl$_2$). Yield 161 mg (0.41 mmol, 45%); mp 72−75 °C; *R$_f$* 0.38 (10% MeOH in CH$_2$Cl$_2$). The product was recrystallized from CH$_2$-Cl$_2$; ^{1}H NMR (DMSO-*d$_6$*) δ 8.38 (s, 1H, H-8), 7.39 (s, 2H, NH$_2$), 5.85 (d, *J* = 6.18 Hz, 1H, H-1′), 5.49 (d, *J* = 6.52 Hz, 1H, OH-2′), 5.32 (d, *J* = 4.81 Hz, 1H, OH-3′), 4.71−4.69 (m, 1H, H-2′), 4.11−4.00 (m, 1H, H-3′), 4.11−4.00 (m, 1H, H-4′), 2.85 (pd, *J* = 7.55 Hz, 2H, H-5′), 2.49−2.40 (m, 2H, SCH$_2$), 2.49−2.40 (m, 2H, ≡CCH$_2$), 1.52−1.43 (m, 4H, ≡CCH$_2$C*H$_2$*C*H$_2$*), 1.14 (t, *J* = 7.20 Hz, 3H, SCH$_2$C*H$_3$*), 0.90 (t, *J* = 7.21 Hz, 3H, CH$_3$), MS *m/z* 392 (M+H)$^+$; Anal. (C$_{18}$H$_{25}$N$_5$O$_3$S·0.14CH$_2$Cl$_2$) C, H, N.

2-(1-Hexynyl)-5′-*S*-propyl-5′-thioadenosine (39). The reaction was carried out with 2-iodo-5′-*S*-propyl-5′-thioadenosine (**35**, 500 mg, 1.11 mmol). The mixture was purified by column chromatography (eluent CH$_2$Cl$_2$ to 10% MeOH in CH$_2$-Cl$_2$). Yield 320 mg (0.79 mmol, 71%), mp 68−71 °C; *R$_f$* 0.30 (5% MeOH in CH$_2$Cl$_2$). The product was recrystallized from CH$_2$Cl$_2$; ^{1}H NMR (DMSO-*d$_6$*) δ 8.38 (s, 1H, H-8), 7.39 (s, 2H, NH$_2$), 5.86 (d, *J* = 6.18 Hz, 1H, H-1′), 5.50 (d, *J* = 5.83 Hz, 1H, OH-2′), 5.31 (d, *J* = 4.81 Hz, 1H, OH-3′), 4.73 (q, *J* = 5.15 Hz, 1H, H-2′), 4.10 (q, *J* = 4.81 Hz, 1H, H-3′), 4.01−3.99 (m, 1H, H-4′), 2.86 (t, *J* = 5.26 Hz, 2H, H-5′), 2.49−2.41 (m, 2H, ≡CCH$_2$), 2.49−2.41 (m, 2H, SCH$_2$), 1.61−1.44 (m, 4H, ≡ CCH$_2$C*H$_2$*C*H$_2$*), 1.61−1.44 (m, 2H, SCH$_2$C*H$_2$*), 0.94−0.83 (m, 6H, 2 × CH$_3$); MS *m/z* 406 (M+H)$^+$; Anal. (C$_{19}$H$_{27}$N$_5$O$_3$S·0.12-CH$_2$Cl$_2$) C, H, N.

2-(1-Hexynyl)-5′-*S*-isopropyl-5′-thioadenosine (40). The reaction was carried out with 2-iodo-5′-*S*-isopropyl-5′-thioadenosine (**36**, 500 mg, 1.11 mmol). The mixture was purified by column chromatography (eluent CH$_2$Cl$_2$ to 10% MeOH in CH$_2$Cl$_2$). Yield 306 mg (0.75 mmol, 68%), mp 77−81 °C; *R$_f$* 0.28 (5% MeOH in CH$_2$Cl$_2$). The product was recrystallized from CH$_2$Cl$_2$; ^{1}H NMR (DMSO-*d$_6$*) δ 8.39 (s, 1H, H-8), 7.40 (bs, 2H, NH$_2$), 5.86 (d, *J* = 5.84 Hz, 1H, H-1′), 5.50 (d, *J* = 6.17 Hz, 1H, OH-2′), 5.32 (d, *J* = 4.81 Hz, 1H, OH-3′), 4.74 (q, *J* = 5.83 Hz, 1H, H-2′), 4.12 (q, *J* = 3.77 Hz, 1H, H-3′), 4.09−3.98 (m, 1H, H-4′), 2.95−2.88 (m, 2H, H-5′), 2.95−2.88 (m, 1H, CH), 2.40 (t, *J* = 6.52 Hz, 2H, ≡CCH$_2$), 1.53−1.45 (m, 4H, C*H$_2$*C*H$_2$*),

1.18 (d, *J* = 6.86 Hz, 6H, (CH$_3$)$_2$), 0.91 (t, *J* = 7.21 Hz, 3H, CH$_3$); MS *m/z* 406 (M+H)$^+$; Anal. (C$_{19}$H$_{27}$N$_5$O$_3$S·0.17CH$_2$Cl$_2$) C, H, N.

General Method for the Introduction of a 2-Hydrazino Group at Derivatives 1 and 33−36 To Obtain 2 and 41−44. A pressure tube was charged with the appropriate 5′-*S*-alkyl-2-iodo-5′-thioadenosine (1.37 mmol) and 10 mL of 2-propanol. To the obtained suspension was added hydrazine monohydrate (14.0 mmol, 678 μL) in 1 mL of 2-propanol. The mixture was heated to 80 °C, and after 30 min a clear solution was obtained. The solution was heated overnight at 80 °C. A white precipitate had appeared, and the mixture was cooled to room temperature under vigorous stirring. The white powder was filtered and dried.

2-Hydrazinoadenosine (2).[7] The reaction was carried out with 2-iodoadenosine (**1**, 500 mg, 1.27 mmol). Yield 310 mg (1.04 mmol, 82%)

2-Hydrazino-5′-*S*-methyl-5′-thioadenosine (41). The reaction was carried out with 2-iodo-5′-*S*-methyl-5′-thioadenosine (**33**, 540 mg, 1.28 mmol). Yield 240 mg (0.73 mmol, 57%), ^{1}H NMR (DMSO-*d$_6$*) δ 7.94 (s, 1H, H-8), 7.30 (bs, 1H, NH), 6.82 (bs, 2H, NH$_2$), 5.77 (d, *J* = 5.83 Hz, 1H, H-1′), 4.70 (t, *J* = 5.83 Hz, 1H, H-2′), 4.13 (t, *J* = 3.78 Hz, 1H, H-3′), 4.01−3.92 (m, 1H, H-4′), 2.81 (t, *J* = 7.20 Hz, 2H, H-5′), 2.04 (s, 3H, SCH$_3$).

5′-Ethyl-2-hydrazino-5′-thioadenosine (42). The reaction was carried out with 5′-*S*-ethyl-2-iodo-5′-thioadenosine (**34**, 600 mg, 1.37 mmol). Yield 358 mg (1.05 mmol, 76%), ^{1}H NMR (DMSO-*d$_6$*) δ 7.94 (s, 1H, H-8), 7.31 (bs, 1H, NH), 6.83 (bs, 2H, NH$_2$), 5.77 (d, *J* = 5.83 Hz, 1H, H-1′), 4.69 (t, *J* = 5.49 Hz, 1H, H-2′), 4.13 (t, *J* = 4.11 Hz, 1H, H-3′), 3.95−3.93 (m, 1H, H-4′), 2.84 (t, *J* = 6.52 Hz, 2H, H-5′), 2.49−2.47 (m, 2H, SCH$_2$), 1.13 (t, *J* = 7.56 Hz, 3H, CH$_3$).

2-Hydrazino-5′-S-propyl-5′-thioadenosine (43). The reaction was carried out with 2-iodo-5′-*S*-propyl-5′-thioadenosine (**35**, 700 mg, 1.55 mmol). Yield 468 mg (1.32 mmol, 85%), ^{1}H NMR (DMSO-*d$_6$*) δ 7.95 (s, 1H, H-8), 7.31 (bs, 1H, NH), 6.84 (bs, 2H, NH$_2$), 5.77 (d, *J* = 5.15 Hz, 1H, H-1′), 4.71 (t, *J* = 5.84 Hz, 1H, H-2′), 4.20−4.10 (m, 1H, H-3′), 4.07−3.91 (m, 1H, H-4′), 2.83 (t, *J* = 6.18 Hz, 2H, H-5′), 2.49−2.44 (m, 2H, SCH$_2$), 1.50 (q, *J* = 7.20 Hz, 2H, C*H$_2$*CH$_3$), 0.88 (t, *J* = 7.55 Hz, 3H, CH$_3$).

2-Hydrazino-5′-*S*-isopropyl-5′-thioadenosine (44). The reaction was carried out with 2-iodo-5′-*S*-isopropyl-5′-thioadenosine (**36**, 700 mg, 1.55 mmol). Yield 452 mg (1.27 mmol, 82%), ^{1}H NMR (DMSO-*d$_6$*) δ 7.97 (s, 1H, H-8), 7.37 (bs, 1H, NH), 6.86 (bs, 2H, NH$_2$), 5.78 (d, *J* = 5.49 Hz, 1H, H-1′), 4.70 (t, *J* = 5.49 Hz, 1H, H-2′), 4.16 (t, *J* = 4.46 Hz, 1H, H-3′), 3.95 (q, *J* = 3.78 Hz, 1H, H-4′), 3.06−2.77 (m, 2H, H-5′), 3.06−2.77 (m, 1H, CH), 1.17 (d, *J* = 6.86 Hz, 6H, 2 × CH$_3$).

General Procedure To Convert the 2-Hydrazino Group of Derivatives 2 and 41−44 into a 2-(*N*-3-Methyl-1-butylidenehydrazino) Group in Derivatives 3 and 45−48. A pressure tube was charged with the appropriate 5′-*S*-alkyl-2-hydrazino-5′-thioadenosine (1.05 mmol) and 8 mL of methanol. A total of 1.2 molar equiv (1.40 mmol, 146 μL) of isovaleraldehyde was added, and the mixture was refluxed for 18 h. The mixture was concentrated in vacuo and purified by column chromatography.

2-(*N*-3-Methyl-1-butylidenehydrazino)adenosine (3).[7] The reaction was carried out with 2-hydrazinoadenosine (**2**, 310 mg, 1.04 mmol). Yield 274 mg (0.75 mmol, 72%), mp 176−179 °C; *R$_f$* 0.20 (10% MeOH in CH$_2$Cl$_2$). The product was recrystallized from MeOH.

2-(*N*-3-Methyl-1-butylidenehydrazino)-5′-*S*-methyl-5′-thioadenosine (45). The reaction was carried out with 2-hydrazino-5′-*S*-methyl-5′-thioadenosine (**41**, 240 mg, 0.73 mmol). The mixture was purified by column chromatography (eluent 10% MeOH in CH$_2$Cl$_2$). Yield 210 mg (0.53 mmol, 73%), mp 122−125 °C; *R$_f$* 0.25 (10% MeOH in CH$_2$Cl$_2$). The product was recrystallized from CH$_2$Cl$_2$; ^{1}H NMR (DMSO-*d$_6$*) δ 10.02 (bs, 1H, NH), 7.98 (s, 1H, H-8), 7.33 (t, *J* = 5.49 Hz, 1H, N= CH), 6.91 (bs, 2H, NH$_2$), 5.77 (d, *J* = 6.17 Hz, 1H, H-1′), 5.49− 5.41 (m, 1H, OH-2′), 5.24−5.22 (m, 1H, OH-3′), 4.75−4.71 (m,

1H, H-2′), 4.10−4.05 (m, 1H, H-3′), 4.05−3.95 (m, 1H, H-4′), 2.91−2.83 (m, 2H, H-5′), 2.91−2.83 (m, 1H, C*H*(CH$_3$)$_2$), 2.12−2.08 (m, 2H, CH$_2$), 2.04 (s, 3H, SCH$_3$), 0.91 (t, J = 6.87 Hz, 6H, CH$_3$), MS *m/z* 396 (M+H)$^+$; Anal. (C$_{16}$H$_{25}$N$_7$O$_3$S·0.33CH$_2$-Cl$_2$) C, H, N.

5′-*S*-Ethyl-2-(*N*-3-methyl-1-butylidenehydrazino)-5′-thioadenosine (46). The reaction was carried out with 5′-*S*-ethyl-2-hydrazino-5′-thioadenosine (**42**, 358 mg, 1.05 mmol). The mixture was purified by column chromatography (eluent 10% MeOH in CH$_2$Cl$_2$). Yield 193 mg (0.47 mmol, 45%), mp 106−109 °C; ^{1}H NMR (DMSO-d_6) δ 10.32 (bs, 1H, NH), 8.02 (s, 1H, H-8), 7.25−7.09 (m, 1H, N=CH), 6.91 (bs, 2H, NH$_2$), 5.79 (d, J = 6.16 Hz, 1H, H-1′), 5.50−5.36 (m, 1H, OH-2′), 5.28−5.16 (m, 1H, OH-3′), 4.79−4.68 (m, 1H, H-2′), 4.19−4.11-(m, 1H, H-3′), 4.06−3.95 (m, 1H, H-4′), 2.98−2.80 (m, 3H, H-5′, C*H*(CH$_3$)$_2$), 2.49−2.47 (m, 2H, SCH$_2$), 2.11 (t, J = 6.41 Hz, 2H, CHC*H*$_2$), 1.15 (t, J = 9.30 Hz, 3H, SCH$_2$C*H*$_3$), 0.94−0.83 (m, 6H, 2 × CH$_3$); MS *m/z* 410 (M+H)$^+$; HPLC Alltima C18 5 μm column (150 mm × 4.6 mm), reversed phase, flow 1 mL/min. System A: 20−100% MeOH in H$_2$O in 40 min; retention time: 25.31 min. System B: 20−100% CH$_3$CN in H$_2$O in 35 min; retention time: 5.59 min.

2-(*N*-3-Methyl-1-butylidenehydrazino)-5′-*S*-propyl-5′-thioadenosine (47). The reaction was carried out with 2-hydrazino-5′-*S*-propyl-5′-thioadenosine (**43**, 468 mg, 1.32 mmol). The mixture was purified by column chromatography (eluent 10% MeOH in CH$_2$Cl$_2$). Yield 364 mg (0.86 mmol, 65%), mp 150−152 °C; ^{1}H NMR (DMSO-d_6) δ 10.02 (bs, 1H, NH), 7.98 (s, 1H, H-8), 7.34 (t, J = 5.80 Hz, 1H, N=CH), 6.91 (bs, 2H, NH$_2$), 5.76 (d, J = 6.16 Hz, 1H, H-1′), 5.43 (pd, J = 4.55 Hz, 1H, OH-2′), 5.21−5.19 (m, 1H, OH-3′), 4.75 (q, J = 5.28 Hz, 1H, H-2′), 4.11 (q, J = 3.25 Hz, 1H, H-3′), 3.96 (q, J = 2.76 Hz, 1H, H-4′), 2.96−2.80 (m, 2H, H-5′), 2.96−2.80 (m, 1H, C*H*(CH$_3$)$_2$), 2.49−2.47 (m, 2H, SCH$_2$), 2.09 (t, J = 6.52 Hz, 2H, CHC*H*$_2$), 1.48 (q, J = 7.29 Hz, 2H, SCH$_2$C*H*$_2$), 0.94−0.83 (m, 9H, 3 × CH$_3$), MS *m/z* 424 (M+H)$^+$; Anal. (C$_{18}$H$_{29}$N$_7$O$_3$S· 0.72H$_2$O) C, H, N.

2-(*N*-3-Methyl-1-butylidenehydrazino)-5′-*S*-isopropyl-5′-thioadenosine (48). The reaction was carried out with 2-hydrazino-5′-*S*-isopropyl-5′-thioadenosine (**44**, 452 mg, 1.27 mmol). The mixture was purified by column chromatography (eluent 10% MeOH in CH$_2$Cl$_2$). Yield 350 mg (0.83 mmol, 65%), mp 156−158 °C; ^{1}H NMR (DMSO-d_6) δ 10.03 (bs, 1H, NH), 7.99 (s, 1H, H-8), 7.33 (t, J = 5.86 Hz, 1H, N=CH), 6.93 (bs, 2H, NH$_2$), 5.76 (d, J = 6.07 Hz, 1H, H-1′), 5.45 (d, J = 6.07 Hz, 1H, OH-2′), 5.22 (d, J = 4.80 Hz, 1H, OH-3′), 4.71 (q, J = 5.37 Hz, 1H, H-2′), 4.12 (q, J = 3.38 Hz, 1H, H-3′), 3.98−3.92 (m, 1H, H-4′), 2.94 (t, J = 6.38 Hz, 1H, SCH), 2.86 (q, J = 6.83 Hz, 2H, H-5′), 2.08 (t, J = 6.62 Hz, 2H, CHC*H*$_2$), 1.86−1.76 (m, 1H, C*H*(CH$_3$)$_2$), 1.15 (d, J = 6.70 Hz, 6H, SCH(C*H*$_3$)$_2$)), 0.90 (d, J = 6.54 Hz, 6H, 2 × CH$_3$); MS *m/z* 424 (M+H)$^+$; HPLC Alltima C18 5 μm column (150 mm × 4.6 mm), Reversed phase, flow 1 mL/min. System A: 20−100% MeOH in H$_2$O in 40 min; retention time: 27.40 min. System B: 20−100% CH$_3$-CN in H$_2$O in 35 min; retention time: 11.34 min.

Radioligand Binding Studies. Measurements with [^{3}H]-DPCPX in the absence of GTP were performed according to a protocol published previously.[36] Adenosine A$_{2A}$ receptor affinities were determined according to Gao et al.[37] Adenosine A$_3$ receptor affinities were determined essentially as described.[4,38] Briefly, assays were performed in 50/10/1 buffer (50 mM Tris/10 mM MgCl$_2$/1 mM ethylenediaminetetraacetic acid (EDTA) and 0.01% 3-([3-cholamidopropyl]-dimethylammonio)-1-propanesulfonate (CHAPS)) in glass tubes and contained 50 μL of a HEK 293 cell membrane suspension (10−30 μg), 25 μL [^{125}I]AB MECA (final concentration 0.15 nM), and 25 μL of ligand. Incubations were carried out for 1 h at 37 °C and were terminated by rapid filtration over Whatman GF/B filters, using a Brandell cell harvester (Brandell, Gaithersburg, MD). Tubes were washed three times with 3 mL of buffer. Radioactivity was determined in a Beckman 5500B γ-counter. Nonspecific binding was determined in the presence of 10^{-5} M *R*-PIA.

cAMP Assay A$_{2A}$. CHO cells expressing human adenosine A$_{2A}$ receptors were grown overnight as a monolayer in 24 wells tissue culture plates (400 μL/well; 2 × 10^5 cells/well). cAMP generation was performed in Dulbecco's Modified Eagles Medium (DMEM)/*N*-2-hydroxyethylpiperazin-*N*-2-ethane-sulfonic acid (HEPES) buffer (0.60 g HEPES/50 mL DMEM pH 7.4). To each well, washed three times with DMEM/HEPES buffer (250 μL), were added 100 μL of DMEM/HEPES buffer, 100 μL of adenosine deaminase (final concentration 5 IU/mL), and 100 μL of a mixture of rolipram and cilostamide (final concentration 50 μM each). After incubation for 40 min at 37 °C, 100 μL of agonist was added. Here, the full agonist CGS 21680 or compounds **1**, **3**, **4**, **33−40**, or **45−48** were used. After 15 min at 37 °C, the reaction was terminated by removing the medium and adding 200 μL of 0.1 M HCl. Wells were stored at −20 °C until assay.

cAMP Assay A$_3$. CHO cells expressing the human adenosine A$_3$ receptor were grown overnight as a monolayer in 24-well tissue culture plates (400 μL/well; 2 × 10^5 cells/well). cAMP generation was performed in Dulbecco's Modified Eagles Medium (DMEM)/*N*-2-hydroxyethylpiperazin-*N*-2-ethansul-fonic acid (HEPES) buffer (0.60 g HEPES/50 mL DMEM pH 7.4). To each well, washed three times with DMEM/HEPES buffer (250 μL), were added 100 μL of adenosine deaminase (final concentration 5 IU/mL), 100 μL of a mixture of rolipram and cilostamide (final concentration 50 μM each), and 100 μL of agonist (final concentration approximately 100× the K_i value). After incubation for 40 min at 37 °C, 100 μL forskolin (final concentration 10 μM) was added. After 15 min at 37 °C, the reaction was terminated by removing the medium and adding 200 μL 0.1 M HCl. Wells were stored at −20 °C until assay. The amounts of cAMP were determined after a protocol with cAMP binding protein[39] with the following minor modifications. As a buffer was used 150 mM K$_2$HPO$_4$/10 mM EDTA/0.2% Bovine Serum Albumine (BSA) at pH 7.5. Samples (20 μL + 30 μL 0.1 M HCl) were incubated for at least 2.5 h at 0 °C before filtration over Whatman GF/B filters. Filters were additionally rinsed with 2 × 2 mL of TrisHCl buffer (pH 7.4, 4 °C). Filters were counted in Packard Emulsifier Safe scintillation fluid (3.5 mL) after 24 h of extraction.

Data Analysis. Apparent K_i and EC$_{50}$ values were computed from the displacement curves by nonlinear regression of the competition curves with the software package Prism (Graph Pad, San Diego, CA).

Supporting Information Available: NMR data for compounds **2−4**, **17−36**. This material is available free of charge via the Internet at http://pubs.acs.org.

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